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(54) Title: INHIBITORS OF LFA-1 BINDING TO ICAMs AND USES THEREOF

(57) Abstract

Novel compounds are disclosed that inhibit binding of LFA-1 to a natural ligand binding partner. Uses of the compounds are also provided.

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INHIBITORS OF LFA-1 BINDING TO ICAMs AND USES THEREOF

BACKGROUND

The leukocyte function-associated antigen (LFA-1, CD11a/CD18) is a leukocyte-specific β_2 integrin that participates in cell/cell adhesion. Binding activity of LFA-1 is essential to leukocyte extravasation from circulation to a site of injury in an inflammatory response. Three principle ligands are known to bind LFA-1, ICAM-1, ICAM-2, and ICAM-3, which are intercellular adhesion molecules that play an important role in localizing leukocyte adhesion to endothelial cells at a site of injury. ICAM-4 and ICAM-5 have also been reported to bind LFA-1. Most leukocytes constitutively express LFA-1, but ligand binding requires activation believed to induce a conformational change and increased avidity ligand binding. For example, ICAM-1 is normally expressed at low levels on the endothelium, however, injury-induced inflammatory mediators promote enhanced surface expression in cells at the site of the injury which, in turn, promotes localized leukocyte adhesion through binding with activated LFA-1.

The structure of LFA-1 includes distinct intracellular and extracellular domains that are believed to participate and/or regulate ICAM binding. Of particular interest is a region in the α_L chain of approximately 200 amino acids, designated the I domain, that is found in all β_2 integrins, as well as many other proteins. Evidence suggests that the I domain is essential to LFA-1 binding to ICAM-1 and 3. For example, anti-LFA-1 blocking monoclonal antibodies have been mapped to epitopes within the I domain. In addition, recombinant I domain polypeptide fragments have been shown to inhibit integrin-mediated adhesion and bind ICAM-1. Within the I domain of LFA-1 (and other proteins) is a single metal ion dependent adhesion site (MIDAS) that preferentially binds manganese or magnesium ions. Binding of either cation is required for ligand interaction and is believed to induce conformational changes in LFA-1 necessary for binding. Cation binding may therefore be a regulatory mechanism that responds to changes in the extracellular leukocyte environment. This hypothesis is supported by the observation that calcium ion

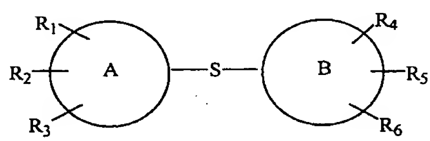
binding actually inhibits LFA-1 interaction with ICAM-1. Indeed, it has been proposed that an inactive LFA-1 conformation results from calcium binding, and that replacement of the calcium ion with a manganese or magnesium ion is a step required for LFA-1 activation [Griggs, *et al., J. Biol. Chem. 273*:22113-22119 (1998)]. Other factors have also been shown to induce LFA-1 activation, including T cell receptor engagement, cytokine stimulation, and *in vitro* PMA stimulation.

In practical terms, the identification of LFA-1/ICAM binding sites provides targets to modulate leukocyte inflammatory responses. Numerous antibodies have been isolated that are capable of inducing LFA-1 activation [see, for example, Landis, *et al., J. Cell Biol. 120*:1519-1527 (1993)] or, for example, preventing ICAM-1 interaction [see for example, Randi and Hogg, *J. Biol. Chem. 269*:12395-12398 (1994)]. The previous identification of anti-LFA-1 activating antibodies that recognize multiple and distinct extracellular epitopes suggests the existence of more than one regulatory region, presumably independent of cytoplasmic signaling. Localization of LFA-1 sites that bind ICAM-1 has been investigated through use of chimeric LFA-1 α subunit proteins comprising human and murine components [Huang and Springer, *J. Biol. Chem. 270*:19008-19016 (1995)]. Studies have indicated that residues that coordinate cation binding and residues proximal to the site are essential for binding ICAM-1 at a relatively flat interface. More precise delineation of the extracellular regulatory region(s) and the contact points for ICAM-1 binding will permit design of efficient modulators.

Thus there exists a need in the art to precisely identify regulatory regions for proteins that participate in inflammatory responses, and in particular LFA-1 and ICAMs that bind LFA-1. Determining the tertiary (or quartenary) structure of a protein can identify potential regulatory regions to permit the rational design of biologically compatible small molecules for therapeutic and prophylactic intervention for inflammatory disorders. There further exists a need in the art to identify compounds that can inhibit LFA-1 binding to ICAMs that can be used in the treatment of inflammatory disorders.

SUMMARY OF THE INVENTION

The present invention is directed to compounds that bind to a novel regulatory site in the I domain of LFA-1, and thereby inhibit LFA-1 binding to ICAMs that bind LFA-1. The present invention therefore also provides methods to regulate leukocyte adhesion to endothelial cells. Compounds of the invention are useful for the treatment of pathologies, such as those associated inflammatory diseases, autoimmune diseases, tumor metastasis, allograft rejection and reperfusion injury. In particular, the present invention is directed to diaryl sulfides of general structural formula (I), a pharmaceutically acceptable salt, or prodrug thereof, and to the use of diaryl sulfides, and particularly compounds of formula (I), to inhibit LFA-1 binding to an ICAM that binds LFA-1.



(I)

wherein A and B, independently, are aryl groups selected from the group consisting of 5- and 6-membered aromatic rings, including, but not limited to, phenyl, thienyl, furyl, pyrimidinyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrrolyl, and pyridazinyl;

R_1, R_2 and R_3, independently, are selected from the group consisting of

hydrogen,

-R_a, wherein R_a is hydrogen or an alkyl group containing one to six saturated straight or branched chain carbon atoms (C_{1-6} alkyl),

-O-R_a,

-halo, wherein halo is Cl, F, Br, or I,

-NR_bR_c, where R_b and R_c, independently, are H, C_{1-6} alkyl, or -CH_2-aryl,

-NO_2,

-C(=O)R_a,

-CN,

-perfluoroR_a, such as trifluoromethyl,

-N-C(=O)R_a,

-$(CH_2)_n$-NR_bR_c, wherein n is an integer 1 to 6,

a 5- or 6-membered heterocyclic ring, either aliphatic or aromatic, containing one or more of O, N, or S, optionally substituted, such as morpholino, and

-S-aryl, wherein aryl is a 5- or 6-membered aromatic ring, optionally substituted;

and R_4, R_5 and R_6, independently, are selected from the group consisting of

hydrogen,

-R_a,

-O-R_a,

-halo,

-NR_bR_c,

-NO_2,

-C(=O)R_a,

-CN,

-perfluoroR_a,

-N-C(=O)R_a,

-$(CH_2)_n$-NR_bR_c, and

-a 5- or 6-membered heterocyclic ring, aliphatic or aromatic, containing one or more of O, N, or S, and optionally substituted,

-S-aryl, or wherein

R_4 and R_5 are taken together to form a 5- or 6-membered aromatic ring, optionally containing one or more of O, N, or S in the ring, optionally substituted.

Examples of novel negative regulators of LFA-1 binding to ICAMs, include, but are not limited to the compounds presented in Table I.

Table I

Exemplary Negative Regulators

3-Chloro-4-(2-chlorophenylsulfanyl)-phenylamine hydrochloride
4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide
3-Chloro-4-(2-naphthylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4,5-trichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
3-Methoxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine
5-Amino-2-(2,3-dichlorophenylsulfanyl)-acetophenone hydrochloride
4-(2,3-dichlorophenylsulfanyl)-phenylamine
3-Chloro-4-(1-naphthylsulfanyl)-phenylamine hydrochloride
3-Methyl-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene
4-Methylamino-2,2',4'-trichlorodiphenylsulfide
3-Bromo-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole
4-Amino-2-chlorophenyl-(2'4'-dimethylphenyl)-sulfide hydrochloride
2,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide hydrochloride
4-Amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide
Bis-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide
4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide
2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide
2-Chloro-4-amino-5-N-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine sulfide
4-Aminomethyl-2-chlorophenyl-(2', 4'-dichlorophenyl)-sulfide
4,5-Dichloro-2-(2,4-dichlorophenylsulfanyl)-phenylamine
3,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine
2,3-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine
4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide
5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone oxime
5-Trifluoromethyl-2-phenylsulfanyl-benzonitrile
1-(3,5-dichlorophenyl)-3-phenylsulfanyl-pyrrolidine-2,5-dione
Bis-2,4,6-Trinitrophenyl-sulfide
2-Methyl-1-(2-*o*-tolylsulfanyl-phenyl)-1*H*-pyrrole
3-[2-(4-Chloro-2-nitro-phenylsulfanyl)-phenylamino-3*H*-isobenzofuran-1-one
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
2-Nitro-4-chlorophenyl-(2'aminophenyl)-sulfide
6-Amino-2-chlorophenyl-(4'-methylphenyl)-sulfide
4-Nitrophenyl-(2'-chlorophenyl)-sulfide
2, 4-Dinitrophenyl-(4'-chlorophenyl)-sulfide
4-Aminophenyl-(2'-chlorophenyl)-sulfide
2, 4-Diaminophenyl-(4'-isopropylphenyl)-sulfide
4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine-sulfide

The compounds as represented by structural formula (I) can be prepared by synthetic methods or by metabolic processes. Preparation of the compounds by metabolic processes include both *in vivo* and *in vitro* processes. Pharmaceutical compositions comprising compounds of the invention are also contemplated.

The invention also provides methods of inhibiting LFA-1 binding to ICAMs that bind LFA-1 comprising the step of contacting LFA-1 with a diaryl sulfide, and preferably a compound of structural formula I. Likewise, the invention provides methods of inhibiting leukocyte adhesion to endothelial cells comprising the step of contacting leukocytes expressing LFA-1 with a diaryl sulfide, and preferably a compound of the structural formula (I). The invention also comprehends methods for treating an inflammatory disorder comprising the steps of administering to a mammal an amount of a pharmaceutical composition of the invention sufficient to inhibit binding of LFA-1 to a naturlal ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1. The invention also comprehends methods for treating an inflammatory disorder arising from LFA-1 binding to a natural ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1, comprising administering to a mammal in need thereof a compound that competes with 3-chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine for binding to LFA-1 in an amount sufficient to inhibit binding of the natural ligand to LFA-1. In addition, the invention provides methods of ameliorating a pathological condition associated with LFA-1 binding to an ICAM that binds LFA-1 comprising administering to an individual in need thereof an effective amount of a diaryl sulfide, and preferably a compound of the structural formula (I) to inhibit LFA-1 binding to the ICAM.

Examples of inhibitors of the present invention include, but are not limited to, the compounds set out in Table I.

The invention also provides for use of a compound of the invention in the production of a medicament for the treatment of pathologies associated with LFA-1 binding to ICAM-1.

The invention also provides methods to identify a negative regulator of LFA-1 binding to a natural ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1 comprising the steps of: a) contacting LFA-1 with an activator of

LFA-1 binding; b) measuring LFA-1 binding with the natural ligand in the presence and absence of a test compound; and c) identifying the test compound as an inhibitor when decreased LFA-1 binding to the ligand is detected in the presence of the test compound. In one aspect, the activator is crystal violet.

DETAILED DESCRIPTION OF THE INVENTION

An IC_{50} value for a compound is defined as the concentration of the compound required to produce 50% inhibition of a biological activity of interest. As used herein, a negative regulator is defined as a compound characterized by an IC_{50} for inhibition of LFA-1 binding to a natural ligand. Negative regulators of LFA-1 binding are defined to have an IC_{50} of less than about 200 μM, less than about 100 μM, less than about 50 μM, and preferably from about 0.05 μM to 40 μM.

The term "pharmaceutically acceptable carrier" as used herein refers to those prodrugs of compounds of the invention which are suitable for use in contact with recipient animals and having undue toxicity, irritation, allergic response commensurate with a reasonable benefit/risk ratio, and effective for their intended use.

The term "prodrug" as used herein refers to compounds which are rapidly transformed *in vivo* to the parent compound of the above formula, for example, by hydrolysis. A thorough discussion is provided in Higuchi, *et al.*, Prodrugs as Novel Delivery Systems, vol. 14 of the A.C.S.D. Symposium Series, and in Roche (ed), Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated herein by reference. Prodrug design is discussed generally in Hardma, *et al.*, (Eds), Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, New York, New York (1996), pp. 11-16. Briefly, administration of a drug is followed by elimination from the body or some biotransformation whereby biological activity of the drug is reduced or eliminated. Alternatively, a biotransformation process may lead to a metabolic by-product which is itself more active or equally active as compared to the drug initially administered. Increased understanding of these biotransformation processes permits the design of so-called "prodrugs" which,

following a biotransformation, become more physiologically active in an altered state. Prodrugs are therefore pharmacologically inactive compounds which are converted to biologically active metabolites. In some forms, prodrugs are rendered pharmacologically active through hydrolysis of, for example, and ester or amide linkage, often times introducing or exposing a functional group on the prodrug. The thus modified drug may also react with an endogenous compound to form a water soluble conjugate which further increases pharmacological properties of the compound, for example, as a result of increased circulatory half-life.

As another alternative, prodrugs can be designed to undergo covalent modification on a functional group with, for example, glucuronic acid, sulfate, glutathione, amino acids, or acetate. The resulting conjugate may be inactivated and excreted in the urine, or rendered more potent than the parent compound. High molecular weight conjugates may also be excreted into the bile, subjected to enzymatic cleavage, and released back into circulation, thereby effectively increasing the biological half life of the originally administered compound.

Compounds of the invention may exist as stereoisomers where asymmetric or chiral centers are present. Stereoisomers are designated by either "S" or "R" depending on arrangement of substituents around a chiral carbon atom. Mixtures of stereoisomers are contemplated by the invention. Stereoisomers include enantiomers, diastereomers, and mixtures of the two. Individual stereoisomers of compounds of the invention can be prepared synthetically from commercially available starting materials which contain asymmetric or chiral centers or by preparation of racemic mixtures followed by separation or resolution techniques well known in the art. Methods of resolution include (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture by recrystallization or chromatography, and liberation of the optically pure product from the auxiliary; (2) salt formation employing an optically active resolving agent, and (3) direct separation of the mixture of optical enantiomers on chiral chromatographic columns.

Compounds of the present invention include, but are not limited to those embraced by general structural formula (I) above and the compounds set out in

Table I.

The invention also provides pharmaceutical compositions comprising one or more compounds of the invention, preferably further comprising a pharmaceutically acceptable carrier or diluent.

The invention further provides methods for inhibiting LFA-1 binding to an ICAM that binds LFA-1 comprising the step of contacting LFA-1, or an ICAM-binding fragment thereof, with a negative regulator compound; said negative regulator binding the LFA-1 α_L polypeptide, or a fragment thereof, at a site selected from the group consisting of a conformation that binds a diaryl sulfide or a binding site defined by Ile^{259}, Leu^{298}, Ile^{235}, Val^{157}, Leu^{161}, and Ile^{306} of human LFA-1 α_L polypeptide and an LFA-1 domain that binds 3-chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine having the structure described above. Alternatively, the negative regulator binding site on LFA-1 is defined by amino acid residues Ile^{259}, Leu^{298}, Ile^{235}, Val^{157}, Leu^{161}, Ile^{306}, Leu^{302},Tyr^{257}, Leu^{132}, Val^{233}, Val^{130}, and Tyr^{166}. In still another alternative, the negative regulator binding site on LFA-1 is defined by amino acid residues Lys^{287}, Leu^{298}, Ile^{259}, Leu^{302}, Ile^{235}, Val^{157}, Tyr^{257}, Lys^{305}, Leu^{161}, Leu^{132}, Val^{233}, Ile^{255}, Val^{130}, Tyr^{166}, Ile^{306}, Phe^{134}, Phe^{168}, Phe^{153}, Tyr^{307}, Val^{308}, Ile^{309}, Thr^{231}, Glu^{284}, Phe^{285}, Glu^{301}, Met^{154}, Ile^{237}, Ile^{150}, and Leu^{295}. The LFA-1 regulatory binding site is described in co-pending U.S. patent application entitled "LFA-1 Regulatory Binding Site and Uses Thereof", filed April 2, 1999, attorney docket number 27866/35375, Serial Number 09/285,477, incorporated herein by reference in its entirety. In one embodiment, methods of the invention include use of cells expressing either LFA-1 or the ICAM. In methods wherein one of the binding partners is expressed in a cell, the other binding partner is either purified and isolated, in a fluid sample (purified, partially purified, or crude) taken from an individual, or in a cell lysate. The invention also comprehends methods wherein both LFA-1 and the ICAM are expressed in cells. The LFA-1 and ICAM binding partners may be expressed on the same cell type or different cell types. Preferably, the LFA-1 polypeptide is expressed on leukocytes, *i.e.* lymphocytes, monocytes, or granulocytes, and the ICAM polypeptide is expressed on endothelial cells.

The invention also provides methods to inhibit leukocyte adhesion to

endothelial cells comprising the step of contacting said leukocyte with a negative regulator of LFA-1 binding to an ICAM that binds LFA-1, said negative regulator binding an LFA-1 regulatory site selected from the group consisting of a conformation that binds a diaryl sulfide or a binding site defined by Ile^{259}, Leu^{298}, Ile^{235}, Val^{157}, Leu^{161}, and Ile^{306} of human LFA-1 α_L polypeptide or an LFA-1 domain that binds 3-chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine. Alternatively, the diaryl sulfide binding conformation is defined by amino acid residues as described above. *In vivo* and *in vitro* methods are contemplated.

The invention also provides methods to ameliorate a pathology arising from LFA-1 binding to an ICAM comprising the step of administering to an individual in need thereof a negative regulator of LFA-1 binding to the ICAM in an amount effective to inhibit LFA-1 binding to the ICAM, said negative regulator binding to an LFA-1 regulatory site selected from the group consisting of a conformation that binds a diaryl sulfide or a site defined by Ile^{259}, Leu^{298}, Ile^{235}, Val^{157}, Leu^{161}, and Ile^{306} of human LFA-1 or an LFA-1 domain that binds compound 3-chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine.

In a preferred embodiment, methods of the invention include use of a diaryl sulfide compound to inhibit binding of LFA-1 to an ICAM. A preferred method includes use of a compound of general structural formula (I), a pharmaceutically acceptable salt, or prodrug thereof as described above.

Therapeutic Methods

To the extent that leukocyte adhesion to endothelial cells gives rise to a pathological disorder, the invention provides methods to ameliorate pathologies associated with accumulation of leukocytes resulting from LFA-1 binding to an ICAM that binds LFA-1 comprising the step of administering to an individual in need thereof an amount of an inhibitor of LFA-1 binding to the ICAM effective to inhibit LFA-1 binding to the ICAM, said inhibitor binding to LFA-1 at a site presented by amino acid residues Ile^{259}, Leu^{298}, Ile^{235}, Val^{157}, Leu^{161} and Ile^{306}. Exemplary medical conditions include, without limitation, inflammatory diseases, autoimmune diseases, reperfusion injury, myocardial infarction, stroke, hemorrhagic shock, organ transplant,

and the like. Methods of the invention provide for amelioration of a variety of pathologies, including, for example, but not limited to adult respiratory distress syndrome, multiple organ injury syndrome secondary to septicemia, multiple organ injury secondary to trauma; reperfusion injury of tissue, acute glomerulonephritis, reactive arthritis, dermatosis with acute inflammatory components, stroke, thermal injury, Crohn's disease; necrotizing enterocolitis, granulocyte transfusion associated syndrome, and cytokine induced toxicity, and T cell mediated diseases.

Inflammatory cell activation and excessive or unregulated cytokine (e.g., TNFα and IL-1β) production are also implicated in disorders such as rheumatoid arthritis, osteoarthritis, gouty arthritis, spondylitis, thyroid associated ophthalmopathy, Behcet disease, sepsis, septic shock, endotoxic shock, gram negative sepsis, gram positive sepsis, toxic shock syndrome, asthma, chronic bronchitis, allergic respiratory distress syndrome, chronic pulmonary inflammatory disease, such as chronic obstructive pulmonary disease, silicosis, pulmonary sarcoidosis, reperfusion injury of the myocardium, brain, and extremities, fibrosis, cystic fibrosis, keloid formation, scar formation, atherosclerosis, transplant rejection disorders, such as graft vs. host reaction and allograft rejection, chronic glamerulonephritis, lupus, inflammatory bowel disease, such as ulcerative colitis, proliferative lymphocyte diseases, such as leukemia, and inflammatory dermatoses, such as atopic dermatitis, psoriasis, urticaria, uveitis.

Other conditions characterized by elevated cytokine levels include brain injury due to moderate trauma (see *J. Neurotrauma*, *12*, pp. 1035-1043 (1995); *J. Clin. Invest.*, *91*, pp. 1421-1428 (1993)), cardiomyopathies, such as congestive heart failure (see *Circulation*, *97*, pp. 1340-1341 (1998)), cachexia, cachexia secondary to infection or malignancy, cachexia secondary to acquired immune deficiency syndrome (AIDS), ARC (AIDS related complex), fever myalgias due to infection, cerebral malaria, osteoporosis and bone resorption diseases, keloid formation, scar tissue formation, and pyrexia.

The ability of the negative regulators of the invention to treat arthritis can be demonstrated in a murine collagen-induced arthritis model [Kakimoto, *et al. Immunol. 142*:326-337 (1992)], in a rat collagen-induced arthritis model [Knoerzer, *et*

al., *Toxical Pathol.* *25*:13-19 (1997)], in a rat adjuvant arthritis model [Halloran, *et al.*, *Arthritis Rheum* *39*:810-819 (1996)], in a rat streptococcal cell wall-induced arthritis model [Schimmer, *et al.*, *J. Immunol.* *160*:1466-1477 (1998)], or in a SCID-mouse human rheumatoid arthritis model [Oppenheimer-Marks, *et al.*, *J. Clin. Invest* *101*:1261-1272 (1998)].

The ability of the negative regulators to treat Lyme arthritis can be demonstrated according to the method of Gross, *et al.*, *Science*, *218*:703-706, (1998).

The ability of the negative regulators to treat asthma can be demonstrated in a murine allergic asthma model according to the method of Wegner, *et al.*, *Science*, *247*:456-459, (1990), or in a murine non-allergic asthma model according to the method of Bloemen, *et al.*, *Am. J. Respir. Crit. Care Med.* *153*:521-529 (1996).

The ability of the negative regulators to treat inflammatory lung injury can be demonstrated in a murine oxygen-induced lung injury model according to the method of Wegner, *et al.*, *Lung*, *170*:267-279, (1992), in a murine immune complex-induced lung injury model according to the method of Mulligan, *et al.*, *J. Immunol.*, *154*:1350-1363, (1995), or in a murine acid-induced lung injury model according to the method of Nagase, *et al.*, *Am. J. Respir. Crit. Care Med.*, *154*:504-510, (1996).

The ability of the negative regulators to treat inflammatory bowel disease can be demonstrated in a murine chemical-induced colitis model according to the method of Bennett, *et al.*, *J. Pharmacol. Exp. Ther.*, *280*:988-1000, (1997).

The ability of the negative regulators to treat autoimmune diabetes can be demonstrated in an NOD mouse model according to the method of Hasagawa, *et al.*, *Int. Immunol.* *6*:831-838 (1994), or in a murine streptozotocin-induced diabetes model according to the method of Herrold, *et al.*, *Cell Immunol.* *157*:489-500, (1994).

The ability of the negative regulators to treat inflammatory liver injury can be demonstrated in a murine liver injury model according to the method of Tanaka, *et al.*, *J. Immunol.*, *151*:5088-5095, (1993).

The ability of the negative regulators to treat inflammatory glomerular injury can be demonstrated in a rat nephrotoxic serum nephritis model according to the method of Kawasaki, *et al.*, *J. Immunol.*, *150*:1074-1083 (1993).

The ability of the negative regulators to treat radiation-induced enteritis can be demonstrated in a rat abdominal irradiation model according to the method of Panes, *et al.*, *Gastroenterology*, *108*:1761-1769 (1995).

The ability of the negative regulators to treat radiation pneumonitis can be demonstrated in a murine pulmonary irradiation model according to the method of Hallahan, *et al.*, *Proc. Natl. Acad. Sci (USA)*, *94*:6432-6437 (1997).

The ability of the negative regulators to treat reperfusion injury can be demonstrated in the isolated heart according to the method of Tamiya, *et al.*, *Immunopharmacology*, *29*:53-63 (1995), or in the anesthetized dog according to the model of Hartman, *et al.*, *Cardiovasc. Res.* *30*:47-54 (1995).

The ability of the negative regulators to treat pulmonary reperfusion injury can be demonstrated in a rat lung allograft reperfusion injury model according to the method of DeMeester, *et al.*, *Transplantation*, *62*:1477-1485 (1996), or in a rabbit pulmonary edema model according to the method of Horgan, *et al.*, *Am. J. Physiol.* *261*:H1578-H1584 (1991).

The ability of the negative regulators to treat stroke can be demonstrated in a rabbit cerebral embolism stroke model according to the method of Bowes, *et al.*, *Exp. Neurol.*, *119*:215-219 (1993), in a rat middle cerebral artery ischemia-reperfusion model according to the method of Chopp, *et al.*, *Stroke*, *25*:869-875 (1994), or in a rabbit reversible spinal cord ischemia model according to the method of Clark *et al.*, *Neurosurg.*, *75*:623-627 (1991). The ability of the negative regulators to treat cerebral vasospasm can be demonstrated in a rat experimental vasospasm model according to the method of Oshiro, *et al.*, *Stroke*, *28*:2031-2038 (1997).

The ability of the negative regulators to treat peripheral artery occlusion can be demonstrated in a rat skeletal muscle ischemia/reperfusion model according to the method of Gute, *et al.*, *Mol. Cell Biochem.*, *179*:169-187 (1998).

The ability of the negative regulators to treat graft rejection can be demonstrated in a murine cardiac allograft rejection model according to the method of Isobe, *et al.*, *Science*, *255*:1125-1127 (1992), in a murine thyroid gland kidney capsule model according to the method of Talento, *et al.*, *Transplantation*, *55*:418-422 (1993),

in a cynomolgus monkey renal allograft model according to the method of Cosimi, *et al.*, *J. Immunol.*, *144*:4604-4612 (1990), in a rat nerve allograft model according to the method of Nakao, *et al.*, *Muscle Nerve*, *18*:93-102 (1995), in a murine skin allograft model according to the method of Gorczynski and Wojcik, *J. Immunol. 152*:2011-2019, (1994), in a murine corneal allograft model according to the method of He, *et al.*, *Opthalmol. Vis. Sci.*, *35*:3218-3225 (1994), or in a xenogeneic pancreatic islet cell transplantation model according to the method of Zeng, *et al.*, *Transplantation*, *58*:681-689 (1994).

The ability of the negative regulators to treat graft-vs.-host disease (GVHD) can be demonstrated in a murine lethal GVHD model according to the method of Harning, *et al.*, *Transplantation*, *52*:842-845 (1991).

The ability of the negative regulators to treat cancers can be demonstrated in a human lymphoma metastasis model (in mice) according to the method of Aoudjit, *et al.*, *J. Immunol.*, *161*:2333-2338, (1998).

Pharmaceutical Compositions

The present invention also provides pharmaceutical compositions which comprise a diaryl sulfide formulated together with one or more pharmaceutically-acceptable carriers.

The pharmaceutical compositions of the invention can be administered to humans and other animals by any suitable route. For example, the compositions can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or nasally. The term "parenteral" administration as used herein refers to modes of administration which include intravenous, intraarterial, intramuscular, intraperitoneal, intrasternal, intrathecal, subcutaneous and intraarticular injection and infusion.

Pharmaceutical compositions of this invention for parenteral injection comprise pharmaceutically-acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water,

ethanol, polyols (such as glycerol, propylene, glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils (such as olive oils), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like, Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monosterate and gelatin.

In some cases, in order to prolong the effect of the drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous materials with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug from is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such a polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The injectable formulations can be sterilized, for example, by filtration through a bacterial- or viral-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with a least one inert, pharmaceutically-acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders such as, for example, carboxymethylcellulose, gums (*e.g.* alginates, acacia) gelatin, polyvinylpyrrolidone, and sucrose, (c) humectants such as glycerol, (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, (e) solution retarding agents such a paraffin, (f) absorption accelerators such as quaternary ammonium compounds, (g) wetting agents such as, for example, cetyl alcohol and glycerol monosterate, (h) absorbents such as kaolin and bentonite clay, and (I) lubricants such as talc, calcium sterate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredients(s) only, or preferentially, in a part of the intestinal tract, optionally, in a delayed manner. Exemplary materials include polymers having pH sensitive solubility, such as the materials available as Eudragit®. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically-acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and

emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

Compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically-acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

The compounds of the present invention may be used in the form of pharmaceutically-acceptable salts derived from inorganic or organic acids. By

"pharmaceutically-acceptable salt" is meant those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically-acceptable salts are well known in the art. For example, S. M. Berge, *et al.*, describe pharmaceutically-acceptable salts in detail in *J. Pharmaceutical Sciences*, *66*:1 (1977). The salts may be prepared *in situ* during the final isolation and purification of the compounds of the invention or separately by reacting a free base function with a suitable acid. Representative acid addition salts include, but are not limited to acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorolsulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate (isothionate), lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-toluenesulfonate and undecanoate. Examples of acids which may be employed to form pharmaceutically acceptable acid addition salts include inorganic acids as hydrochloric acid, hydrobromic acid, sulphuric acid and phosphoric acid and such organic acids as oxalic acid, maleic acid, succinic acid and citric acid.

Basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialky sulfates like dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; arylalkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

Basic addition salts can be prepared *in situ* during the final isolation and purification of compounds of this invention by reacting a carboxylic acid-containing moiety with a suitable base such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation or with ammonia or organic primary, secondary or tertiary amine. Pharmaceutically-acceptable basic addition salts include, but are not limited to, cations based on alkali metals or alkaline earth metals such as

lithium, sodium, potassium, calcium, magnesium and aluminum salts and the like and nontoxic quaternary ammonia and amine cations including ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine and the like. Other representative organic amines useful for the formation of base addition salts include ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine and the like.

Dosage forms for topical administration of a compound of this invention include powders, sprays, ointments and inhalants. The active compound is mixed under sterile conditions with a pharmaceutically-acceptable carrier and any needed preservatives, buffers, or propellants which may be required. Ophthalmic formulations, eye ointments, powders, and solutions are also contemplated as being within the scope of this invention.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular compound, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired therapeutic effort and to gradually increase the dosage until the desired effect is achieved.

Generally dosage levels of about 0.1 to about 1000 mg, about 0.5 to about 500 mg, about 1 to about 250 mg, about 1.5 to about 100, and preferably of about 5 to about 20 mg of active compound per kilogram of body weight per day are administered orally or intravenously to a mammalian patent. If desired, the effective daily dose may be divided into multiple doses for purposes of administration, e.g., two to four separate doses per day.

The present invention is illustrated by the following examples. Example 1 described a high throughput assay to screen for negative regulators of

LFA-1 binding to an ICAM that binds LFA-1. Example 2 relates to binding assays to evaluate the ability of various compounds to inhibit LFA-1 binding to an ICAM. Example 3 describe synthesis of negative regulators. Example 4 provides results from cell based assays using the negative regulators.

Example 1
High Throughput Screening for LFA-1/ICAM-1 Binding Inhibitors

In an effort to identify inhibitors of LFA-1/ICAM-1 binding, a high throughput screening (HTS) assay was designed to efficiently screen large numbers of chemical compounds in a proprietary library as follows.

Preliminary experiments were carried out in order to define the linear range of LFA-1/ICAM-1 interaction. Recombinant ICAM-1/IgG1 fusion protein (comprising full length ICAM-1) was prepared as described in U.S. Patent Nos. 5,770,686, 5,837,478, and 5,869,262, each of which is incorporated herein by reference. The fusion protein was biotinylated using a kit obtained from Pierce Chemical (Rockford, IL). Biotinylated protein (BioIgICAM-1) concentration was determined by measuring absorbance at 280 nm, and serial dilutions were prepared to give a final concentration range of 50 μg/ml to 0.008 μg/ml. Titration of BioIgICAM-1 was carried out with the protein first aliquoted into wells on an assay plate. Recombinant LFA-1 was added to each well at the same concentration and the experiment (as described below) was carried to completion. The amount of binding was determined for each well, and from a subsequent plot of the results, a single concentration of BioIgICAM-1 was selected for subsequent experiments. In a similar manner, LFA-1 was titrated using the BioIgICAM-1 concentration selected as described above.

On day 1 of the HTS procedure, the capture antibody, *i.e.*, a non-blocking anti-LFA-1 monoclonal antibody (TS2/4.1; ATCC #HB244), was diluted in plate coating buffer (50 mM sodium carbonate/bicarbonate, 0.05% ProClin® 300, pH 9.6) to a final concentration of 2 μg/ml. Immulon® 4 (Dynex Technologies, Chantilly, VA) plate wells were coated with 100 μl diluted antibody solution per well, and incubation was carried out overnight at 4°C. On day 2, the

plates were warmed to room temperature and washed two times with wash buffer (calcium- and magnesium-free phosphate buffered saline, CMF-PBS) with 0.05% Tween®-20). To each well, 200 µl of blocking solution (5% fish skin gelatin in CMF-PBS with 0.05% ProClin® 300) was added, and the blocking incubation was carried out at room temperature for 30 min. The blocking solution was removed by aspiration, and the plates were not washed. LFA-1 was diluted to a final concentration of 1 µg/ml in assay buffer (1% fish skin gelatin and 2 mM $MgCl_2$ in CMF-PBS), and 100 µl was added to each well. Incubation was carried out for one hour, and the plates were washed two times with wash buffer.

A 2X stock solution of BioIgICAM-1 was prepared containing 0.1 µg/ml BioIgICAM-1 and 4 µM crystal violet (found to be an activator of LFA-1/ICAM-1 binding) in Assay Buffer (EG&G Wallac, Gaithersburg, MD). Aliquots (50 µl) of pooled chemicals (22 compounds/pool in 100% DMSO) from the chemical library were added to the wells, followed by addition of 50 µl of the 2X stock of BioIgICAM-1 to provide a final assay volume of 100 µl (containing 2% DMSO). The plates were incubated for one hour at room temperature, and washed once with wash buffer. Europium-labeled streptavidin (Eu-SA; #1244-360, EG&G Wallac) was diluted 1:500 in Assay Buffer, 100 µl of the diluted Eu-SA was added to each well, and the plates were incubated at room temperature for one hour.

Plates were washed eight times with wash buffer, 100 µl of DELFIA® enhancement solution (EG&G Wallac) diluted 1:2, was added to each well, and the plates were shaken for five minutes using a Wallac shaker at fast speed. Plates were read using a Wallac DELFIA® fluorescence reader (fluorimeter). Controls included both positive and negative wells and 50% binding wells established using blocking antibodies, *i.e.*, anti-LFA-1 monoclonal antibody (TS1/22.1, ATCC #HB202) or anti-ICAM-1 monoclonal antibody. Chemical pools in wells showing 50% or greater inhibition of LFA-1 binding to ICAM-1 were identified and the experiment was repeated using individual chemicals from those pools. Inhibitors of LFA-1/ICAM-1 binding were identified, and a further screen was performed to determine dose dependence of the inhibitory activity. Further study of selected compounds was carried out using biochemical and cellular assay techniques.

The compounds were grouped according to common structural features, and it was found that a subset (listed below) of the compounds included a characteristic diaryl sulfide structure.

3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone oxime
5-Trifluoromethyl-2-phenylsulfanyl-benzonitrile
1-(3,5-dichlorophenyl)-3-phenylsulfanyl-pyrrolidine-2,5-dione
Bis-2,4,6-Trinitrophenyl-sulfide
2-Methyl-1-(2-*o*-tolylsulfanyl-phenyl)-1*H*-pyrrole
3-[2-(4-Chloro-2-nitro-phenylsulfanyl)-phenylamino-3*H*-isobenzofuran-1-one
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
2-Nitro-4-chlorophenyl-(2′aminophenyl)-sulfide
6-Amino-2-chlorophenyl-(4′-methylphenyl)-sulfide
4-Nitrophenyl-(2′-chlorophenyl)-sulfide
2, 4-Dinitrophenyl-(4′-chlorophenyl)-sulfide
4-Aminophenyl-(2′-chlorophenyl)-sulfide
2, 4-Diaminophenyl-(4′-isopropylphenyl)-sulfide

In an effort to optimize the negative regulatory capacity of the identified compounds, various diaryl sulfide derivatives were conducted as described below in Example 3. Additional diaryl sulfide derivatives are described in co-pending provisional patent application entitled "Cell Adhesion-Inhibiting Antiinflammatory and Immune Suppressive Compounds" filed April 2, 1999, attorney docket number 6446.US.Z3, Serial Number 09/286,645, incorporated herein by reference in its entirety.

Example 2
Binding Assays

A. ICAM-1/LFA-1 Biochemical Interaction Assay

Compounds that antagonize the interaction between ICAM-1 and LFA-1 can be identified, and their activities quantitated, using both biochemical and cell-based assays. A primary biochemical assay measures the ability of the compound in question to block the interaction between LFA-1 and its adhesion partner ICAM-1, as described below.

In this biochemical assay, 100 µl of anti-LFA-1 antibody at a connection of 5 µg/ml in Dulbecco's phosphate-buffered saline (D-PBS) was used to coat wells of a 96-well microtiter plate overnight at 4°C. The wells were washed twice with wash buffer (CMF-PBS, 0.05% Tween® 20) and blocked by addition of 200 µl of D-PBS containing 5% fish skin gelatin. Recombinant LFA-1 (100 µl of 0.7 µg/ml) in D-PBS was added to each well. Incubation was continued for one hour at room temperature and the wells were washed twice with wash buffer. Serial dilutions of compounds being assayed as LFA-1/ICAM-1 negative regulators, prepared as 10 mM stock solutions in DMSO, were diluted in D-PBS, 2 mM $MgCl_2$, 1% fish skin gelatin and 500 µl of each dilution added to each well, followed by addition of 50 µl of 0.8 µg/ml BioIgICAM-1 to the wells, and the plates were incubated at room temperature for one hour. The wells were then washed twice with wash buffer and 100 µl of Eu-SA (EG&G Wallac) diluted 1:100 in Delfia® assay buffer (EG&G Wallac) were added to the wells. Incubation was carried out for one hour at room temperature. The wells were washed eight times with wash buffer and 100 µl of enhancement solution (EG&G Wallac) was added to each well. Incubation was continued for five minutes with constant mixing. Time-resolved fluorimetry measurements were made using the Victor 1420 Multilabel Counter (EG&G Wallac) and the percent inhibition of each candidate compound was calculated using the following equation:

$$\%inhibition = 100\ X \left\{ 1 - \frac{average\ OD\ w/compound\ minus\ background}{average\ OD\ w/o\ compound\ minus\ background} \right\}$$

where "background" refers to wells that were not coated with anti-LFA-1 antibody.

B. ICAM-1/JY-8 Cell Adhesion Assay

Biologically relevant activity of the compounds in this invention was confirmed using a cell-based adhesion assay that measures the ability of the compounds to block adherence of JY-8 cells (a human EBV-transformed B cell line expressing LFA-1 on its surface) to immobilized ICAM-1, as follows. This assay may be performed with or without added IL-8. For IL8 stimulation of the standard JY-8 cells 30 ng/ml IL-8 was added in the 30 minute incubation at 37°C with the cells.

For measurement of inhibitory activity in the cell-based adhesion assay, 96-well microtiter plates were coated with 70 µl of recombinant ICAM-1/Ig at a concentration of 5 µg/ml in CMF-PBS overnight at 4°C. The wells were washed twice with D-PBS and blocked by addition of 200 µl of D-PBS, 5% fish skin gelatin by incubation for one hour at room temperature. Fluorescent-tagged JY-8 cells (50 µl at 2 x 10^6 cells/ml in RPMI-1640/1% fetal bovine serum (FBS)) were added to the wells. For fluorescent labeling of JY-8 cells, 5 x 10^6 cells, washed once in RPMI 1640, were resuspended in 1 ml RPMI-1640 containing 2 µM Calcein AM (Molecular Probes, OR), were incubated at 37°C for 30 minutes and washed once with RPMI-1640/1% FBS. Dilutions of compounds to be assayed for LFA-1/ICAM-1 antagonistic activity were prepared in RPMI-1640/1% FBS from 10 mM stock solutions in DMSO and 50 µl aliquots were added to duplicate wells. Microtiter plates were incubated for 45 min at room temperature and the wells were washed gently once with RPMI-1640/1% FBS. Fluorescence intensity was measured in a fluorescence plate reader with an excitation wavelength at 495 nm and an emission wavelength at 530 nm. The percent inhibition of a candidate compound at a given concentration was calculated using the following equation:

$$\%inhibition = 100\ X \left\{ 1 - \frac{average\ OD\ w/compound}{average\ OD\ w/o\ compound} \right\}$$

C. ICAM-3/JY-8 Cell Adhesion Assay

Compounds of the present invention have been demonstrated to act via interaction with the integrin LFA-1, specifically by binding to the α_L I domain which is known to be critical for the adhesion of LFA-1 to a variety of cell adhesion molecules. As such, it is expected that these compounds should block the interaction of LFA-1 with other CAMs, and this inhibition has been demonstrated for LFA-1 binding to ICAM-3. Compounds of the present invention were evaluated for the ability to block the adhesion of JY-8 cells to immobilized ICAM-3, as follows.

For measurement of inhibitory activity in the cell-based adhesion assay, 96-well microtiter plates were coated with 50 µl of recombinant ICAM-3/Ig at a concentration of 10 µg/ml in CMF-PBS overnight at 4°C. The wells were washed twice with D-PBS, blocked by addition of 100 µl of D-PBS, 1% bovine serum albumin (BSA) by incubation for one hour at room temperature, and washed once with RPMI-1640/5% heat-inactivated FBS (adhesion buffer). Dilutions of compounds to be assayed for LFA-1/ICAM-3 antagonistic activity were prepared in adhesion buffer from 10 mM stock solutions in DMSO and 100 µl aliquots were added to duplicate wells. JY-8 cells (100 µl at 0.75×10^6 cells/ml in adhesion buffer) were then added to the wells (with or without 30 ng/ml IL-8). Microtiter plates were incubated for 30 min at room temperature, the adherent cells were fixed with 50 µl of 14% glutaraldehyde/D-PBS and incubation carried out for an additional 90 min. The wells were washed gently with dH_2O and 50 µl of dH_2O was added, followed by 50 µl of 1% crystal violet. After five minutes, the plates were washed twice with dH_2O and 225 µl ethanol (EtOH) was added to each well to extract the crystal violet from the cells. Absorbance was measured at 570 nm in an ELISA plate reader. The percent inhibition of a candidate compound was calculated using the following equation:

$$\%inhibition = 100\ X \left\{ 1 - \frac{average\ OD\ w/compound}{average\ OD\ w/o\ compound} \right\}$$

Example 3
Synthesis of Negative Regulators

Synthesis of various diaryl sulfide compounds according to the invention is described below.

General Procedures and Starting Materials

In general, solvents were purchased anhydrous and were not dried or purified further and starting materials were the best commercially available. Thin layer chromatography (TLC) was carried out using E. Merck, silica gel 60 F254, 0.25 mm, glass or aluminum coated plates, or Analtech silica gel uniplates (250 microns of silica). Visualization was achieved by UV. Reported R_fs indicate a single spot detected. Flash chromatography was performed on E. Merck silica gel 60, 230-400 mesh. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker DPX 300 or Varian 300 Gemini 2000 spectrometers. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Coupling constants are in Hz. In other cases, NMR spectra were recorded using a Unity XL-200 spectrometer. Mass spectra were recorded on either a VG 70 SEG instrument at the University of Washington, Department of Medicinal Chemistry, Mass Spectrometry Facility (high resolution) or a Finnigan Mat TSQ 70 spectrometer (low resolution). Only the molecular ions are reported. Elemental analyses were performed by Quantitative Technologies, Inc.

A. <u>General Synthesis Method A: Aminodiarylsulfides</u>

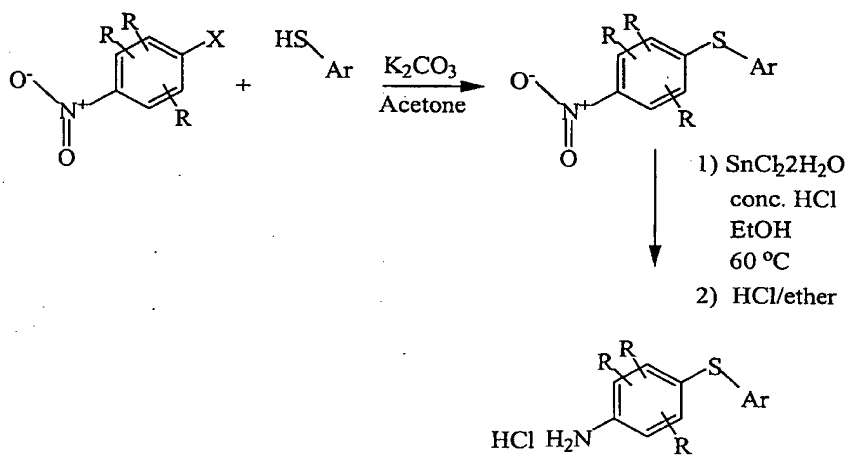
1. <u>General Description of Synthesis Method A</u>

One molar equivalent (eq) of a desired thiolphenol and 1 molar equivalent of the respective nitroaryl compound were placed in a dry flask under nitrogen and dissolved in dry acetone. One and a half molar equivalents anhydrous potassium carbonate (K_2CO_3) was added and the mixture stirred vigorously overnight. The reaction was then diluted with ether, washed with saturated $NaHCO_3$, 3% $NaHSO_4$, and saturated NaCl. The organics were dried over Na_2SO_4 and filtered. Heptane was then added and the solution was concentrated by boiling until most of

the ether was removed. Upon cooling, the nitrodiaryl sulfide crystallized. The product was collected by filtration, washed with pentane, and dried in vacuo.

One molar equivalent of the diaryl sulfide and 5 molar equivalents tin chloride dihydrate were dissolved in ethanol (10 to 30 volumes). The mixture was heated to 60°C in an oil bath and concentrated HCl was added (10 to 30 volumes). After three hours, the reaction was allowed to cool and 20 to 60 volumes of ice was added. The mixture was neutralized to pH 10 - 12 by addition of 5 N NaOH. The mixture was extracted twice with ether and the combined ether extracts were washed with saturated $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4, filtered, and the solvent stripped by rotary evaporation. The resulting solid or oil was taken up in ether, and approximately 5 molar equivalents of 1 N HCl in ether was added drop-wise. The resulting solid was collected by filtration, washed with ether, and dried in vacuo.

The general synthesis method A is diagramed schematically below, wherein X is a halogen, *e.g.*, chlorine.



2. Specific Example of General Synthesis Method A

General Synthesis Method A was employed as specifically described below with the exception that tin granules were employed instead of the preferred tin chloride dihydrate shown in the schematic above.

2,4-Dichlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide

1.54 g (8.60 mmol) 2,4-dichlorothiophenol and 1.65 g (1 eq, 8.60 mmol) 3,4-dichloronitrobenzene were placed in a dry flask under an atmosphere of nitrogen and dissolved in 20 ml dry acetone. 1.78 g (1.5 eq, 12.9 mmol) anhydrous potassium carbonate (K_2CO_3) was added and the mixture was stirred magnetically for 20 hrs. The reaction was then diluted with 150 ml ether and then washed with saturated $NaHCO_3$ (2 x 75 ml), 3% $NaHSO_4$ (2 x 75 ml) and saturated NaCl (2 x 75 ml). The organic phase was then dried over anhydrous Na_2SO_4 and filtered. 50 ml heptane was then added, and the solution was concentrated to approximately 50 ml by boiling on a steam bath. Upon cooling high quality crystals formed. These were collected by filtration, rinsed with three portions of pentane, and dried in vacuo (70°C, 0.05 mm Hg, 2 hrs). 1.43 g (50% yield) of yellow crystals were obtained, mp 145-146 °C, R_f 0.37 (Ethyl acetate [EtAc]/Heptane, 1:20). ^1H NMR ($CDCl_3$) 6.71 (d, J=8.9, 1H), 7.38 (d of d, J_1=2.2, J_2=8.2, 1H), 7.59 (d, J=8.2, 1H), 7.63 (d, J=2.2, 1H), 7.94 (d of d, J_1=2.2, J_2=8.9, 1H), 8.26 (d, J=2.5, 1H). MS (EI) m/z 333 (M+, 98). Anal. Calcd for $C_{12}H_6Cl_3NO_2S$: C, 43.08; H, 1.81; N, 4.19. Found: C, 43.06; H, 1.77; N, 4.02.

3-Chloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine Hydrochloride

0.680 g (2.03 mmol) 2,4-Dichlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide and 0.844 g (3.5 eq, 7.11 mmol) tin granules were slurried in 20 ml concentrated HCl. With vigorous stirring, this was heated to reflux for two days. The mixture was allowed to cool to room temperature and then was diluted with 50 ml ice. While stirring, this mixture was neutralized to pH 10 by the addition of approx. 80 ml 5 N NaOH. This was then extracted with ether (2 x 100 ml). The combined organics were washed with 100 ml saturated

$NaHCO_3$ and 2 x 100 ml saturated NaCl. This was dried over Na_2SO_4 and filtered. This was concentrated on a rotavapor to give a brown oil. The oil was taken up in 50 ml ether, and 4 ml 1 N HCl in ether was then added, drop wise, producing a white solid. This was collected by filtration, rinsed with several portions of ether, and dried (90°C, 3 hrs, 0.05 mm Hg). 0.548 g (79% yield) of a white solid was obtained, mp 183-185°C (dec), R_f 0.33 (EtAc/Heptane 1:2 w/ 1% TEA). ^1H NMR (DMSO-d6) 6.55 (d, J=8.6, 1H), 6.66 (d of d, J_1=2.5, J_2=8.6, 1H), 6.89 (br s, 3H), 6.89 (d, J=2.5, 1H), 7.29 (d, J=2.2, 1H), 7.32 (d, J=2.2, 1H), 7.62 (d, J=2.2, 1H). MS (EI) m/z 303 M+ [100]. Anal. Calcd for $C_{12}H_8Cl_3NS{\cdot}HCl$: C, 42.26; H, 2.66; N, 4.11. Found: C, 42.39; H, 2.57; N, 4.14.

3. Additional Examples of Compounds Prepared by General Synthesis Method A

The following compounds were prepared from commercially available reagents using General Synthesis Method A. The starting thiol and nitroaryl compounds are provided, along with the intermediate (thiol + nitroaryl → intermediate).

3-Chloro-4-(2-chlorophenylsulfanyl)-phenylamine hydrochloride

2-chlorothiophenol + 3,4-dichloronitrobenzene → 4-nitro-2-chlorophenyl-(2′-chlorophenyl)-sulfide

Grey solid, mp 197-198°C (dec), R_f 0.16 (EtAc/Heptane 1:4 w/ 1% TEA). ^1H NMR (DMSO-d6) 6.61 (d of d, J_1=1.9, J_2=7.3, 1H), 6.70 (d of d, J_1=2.4, J_2=8.4, 1H), 6.94 (d, J=2.5, 1H), 7.18 (m, 2H), 7.28 (d, J=8.9, 1H), 7.45 (d of d, J1=1.6, J2=7.6, 1H), 7.78 (br s, 3H). MS (EI) m/z 269 M+ [100]. Anal. Calcd for $C_{12}H_{10}Cl_3NS{\cdot}HCl$: C, 47.22; H, 3.30; N, 4.59. Found: C, 47.34; H, 3.15; N, 4.45.

3-Chloro-4-(2-naphthylsulfanyl)-phenylamine hydrochloride

2-naphthalenethiol + 3,4-dichloronitrobenzene → 4-nitro-2-chlorophenyl-2-naphthyl-sulfide

Off white solid, mp 188°C (dec), R_f 0.16 (EtAc/Heptane 1:4 w/ 1% TEA). ^1H NMR (DMSO-d6) 6.08 (br s, 3H), 6.60 (d of d, J_1=2.4, J_2=8.4, 1H), 6.90

(d, J=2.5, 1H), 7.20 (d of d, J_1=1.9, J_2=8.6, 1H), 7.30 (d, J=8.6, 1H), 7.45 (m, 2H), 7.53 (s, 1H), 7.76 (m, 1H), 7.83 (m, 2H). MS (EI) m/z 285 M+ [100]. Anal. Calcd for $C_{16}H_{12}ClNS{\cdot}HCl$: C, 59.64; H, 4.07; N, 4.35. Found: C, 59.59; H, 4.07; N, 4.09.

3-Chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride

2,3-dichlorothiophenol +3,4-dichloronitrobenzene → 4-nitro-chlorophenyl-(2′,3′-dichlorophenyl)-sulfide

Off white solid, mp 207-209°C (dec), R_f 0.33 (EtAc/Heptane 1:2 w/ 1% TEA). ^{1}H NMR (DMSO-d6) 6.45 (d of d, J_1=1.4, J_2=8.1, 1H), 6.62 (d of d, J_1=2.2, J_2=8.6, 1H), 6.79 (br s, 3H), 6.86 (d, J=2.5, 1H), 7.21 (t, J=7.9, 1H), 7.33 (d, J=8.2, 1H), 7.38 (d of d, J_1=1.4, J_2=8.1, 1H). MS (EI) m/z 303 (M+, 93). Anal. Calcd for $C_{12}H_8Cl_3NS{\cdot}HCl$: C, 42.26; H, 2.66; N, 4.11. Found: C, 42.49; H, 2.56; N, 4.10.

3-Chloro-4-(2,4,5-trichlorophenylsulfanyl)-phenylamine hydrochloride

2,3,4-trichlorothiophenol + 3,4-dichloronitrobenzene →4-nitro-2-chlorophenyl-(2′,4′,5′-trichlorophenyl)-sulfide

Off white solid, mp 192-194°C (dec), R_f 0.33 (EtAc/Heptane 1:2 w/ 1% TEA). ^{1}H NMR (DMSO-d6) 6.46 (br s, 3H), 6.53 (s, 1H), 6.65 (d of d, J_1=2.3, J_2=8.4, 1H), 6.89 (d, J=2.2, 1H), 7.35 (d, J=8.6, 1H), 7.88 (s, 1H). MS (EI) m/z 337 (M+, 73). Anal. Calcd for $C_{12}H_7Cl_4NS{\cdot}HCl$: C, 38.38; H, 2.15; N, 3.73. Found: C, 38.73; H, 2.07; N, 3.60.

3-Methoxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine

2,3-dichlorothiophenol + 2-bromo-5-nitroanisole → 4-nitro-2-methoxyphenyl-(2′,3′-dichlorophenyl)-sulfide

This compound was isolated by crystallization from ether rather than from the formation of the HCl salt. Off white crystals were obtained, mp 166-167°C, R_f 0.17 (EtAc/Heptane 1:2 w/ 1% TEA). ^{1}H NMR (DMSO-d6) 3.67 (s, 3H), 5.75 (br s, 2H), 6.26 (d of d, J_1=1.9, J_2=8.3, 1H), 6.37 (d, J=1.9, 1H), 6.46 (d, J=7.9, 1H), 7.13 (d, J=8.2, 1H), 7.16 (t, J=8.1, 1H), 7.31 (d, J=7.9, 1H). MS (EI) m/z 299 M+ [100]. Anal. Calcd for $C_{13}H_{11}Cl_2NOS$: C, 52.01; H, 3.69; N, 4.67. Found: C, 51.97; H, 3.59;

N, 4.67.

5-Amino-2-(2,3-dichlorophenylsulfanyl)-acetophenone hydrochloride

2,3-dichlorothiophenol + 2-chloronitroacetophenone → 4-nitro-2-acetylphenyl-(2′,3′-dichlorophenyl)-sulfide

Yellow solid, mp 151-153 oC (dec), Rf 0.35 (EtAc/Heptane 1:1 w/ 1% TEA). 1H NMR (DMSO-d6) 2.51 (s, 3H), 7.00-7.09 (m, 3H), 7.34 (t, J=7.9, 1H), 7.41 (br s, 1H), 7.44 (br s, 3H), 7.58 (d, J=7.9, 1H). MS (EI) m/z 311 M+ [100]. Anal. Calcd for C13H11Cl3NOS.HCl: C, 48.23; H, 3.47; N, 4.02. Found: C, 47.78; H, 3.43; N, 3.79.

4-(2,3-dichlorophenylsulfanyl)-phenylamine

2,3-dichlorothiophenol + 4-bromonitrophenol → 4-nitrophenyl-(2′,3′-dichlorophenyl)-sulfide

Off white solid, mp 175°C (dec), R_f 0.31 (EtAc/Heptane 1:2 w/ 1% TEA). ^{1}H NMR (DMSO-d6) 7.13 (d, J=8.2, 2H), 7.26 (t, J=7.8, 1H), 7.42 (d, J=8.6, 2H), 7.47 (d of d, J_1=1.9, J_2=7.8, 1H), 7.76 (d of d, J_1=1.3, J_2=7.9, 1H), 8.04 (br s, 3H). MS (EI) m/z 269 M+ [100]. Anal. Calcd for $C_{12}H_9Cl_2NS{\cdot}HCl$: C, 47.00; H, 3.29; N, 4.57. Found: C, 46.92; H, 3.36; N, 4.27.

3-Chloro-4-(1-naphthylsulfanyl)-phenylamine hydrochloride

1-naphthalenethiol + 3,4-dichloronitrobenzene→ 4-Nitro-2-chlorophenyl-(1-naphthyl)-sulfide

Off white solid, mp 192-194°C, R_f 0.37 (EtAc/Heptane 1:2 w/ 1% TEA). ^{1}H NMR (DMSO-d6) 6.84 (m, 2H), 7.20 (d, J=1.9, 1H), 7.40 (d, J=7.0, 1H), 7.50 (t, J=7.8, 1H), 7.56-7.61 (m, 2H), 7.94 (d, J=8.2, 1H), 7.99 (m, 1H), 8.12-8.15 (m, 5H). MS (EI) m/z 285 M+ [100]. Anal. Calcd for $C_{16}H_{12}ClNS{\cdot}HCl$: C, 59.64; H, 4.07; N, 4.35. Found: C, 59.59; H, 4.19; N, 4.11.

3-Methyl-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride

2,4-dichlorothiophenol + 2-bromo-5-nitrotoluene → 4-Nitro-2-methylphenyl-(2′, 4′-dichlorophenyl)-sulfide

Off white solid, mp 195-197°C, R_f 0.41 (EtAc/Heptane 1:2 w/ 1% TEA). 1H NMR (DMSO-d6) 2.22 (s, 3H), 6.62 (d, J=8.6, 1H), 6.94 (d, J=8.2, 1H), 7.04 (br s, 1H), 7.29 (s, 1H), 7.32 (s, 1H), 7.66 (d, J=2.2, 1H), 7.89 (br s, 3H). MS (EI) m/z 283 M+ [100]. Anal. Calcd for $C_{13}H_{11}Cl_2NS{\cdot}HCl$: C, 48.69; H, 3.77; N, 4.37. Found: C, 48.87; H, 3.73; N, 4.34.

3-Bromo-4-(2,4-dichlorophenylsulfanyl)-phenylamine Hydrochloride

2,4-dichlorothiophenol + 3-bromo-4-chloronitrobenzene → 4-Nitro-2-bromophenyl-(2′, 4′-dichlorophenyl)-sulfide

Off white solid, mp 171-173°C (dec), R_f 0.69 (EtAc/Heptane 1:1 w/ 1% TEA). 1H NMR (DMSO-d6) 6.66 (d, J=8.6, 1H), 6.81 (d of d, J_1=2.2, J_2=8.4, 1H), 7.19 (d, J=2.2, 1H), 7.29 (d, J=8.2, 1H), 7.32 (d of d, J_1=2.2, J_2=8.6, 1H), 7.64 (d, J=2.2, 1H), 7.92 (br s, 3H). MS (EI) m/z 347 (M+, 60). Anal. Calcd for $C_{12}H_8BrCl_2NS{\cdot}HCl$: C, 37.39; H, 2.35; N, 3.63. Found: C, 37.78; H, 2.28; N, 3.61.

2,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine Hydrochloride

2,4-dichlorothiophenol + 2,4,5-trichloronitrophenol → 4-Nitro-2, 5-dichlorophenyl-(2′, 4′-dichlorophenyl)-sulfide

Off white solid, mp 168-176°C, R_f 0.39 (EtAc/Heptane 1:2 w/ 1% TEA). 1H NMR (DMSO-d6) 6.57 (d, J=8.6, 1H), 6.83 (br s, 3H), 7.06 (s, 1H), 7.30 (d of d, J_1= 2.2, J_2=8.6, 1H), 7.55 (s, 1H), 7.63 (d, J=2.2, 1H). MS (EI) m/z 347 (M+, 60). Anal. Calcd for $C_{12}H_7Cl_4NS{\cdot}0.75HCl$: C, 39.34; H, 2.13; N, 3.82. Found: C, 39.34; H, 2.11; N, 3.71.

4,5-Dichloro-2-(2,4-dichlorophenylsulfanyl)-phenylamine

2,4-dichlorothiophenol + 3,4-dichloro-2-fluoronitrobenzene → 4-Nitro-2-chloro-5-fluorophenyl-(2′, 4′-dichlorophenyl)-sulfide

Starting with, 3,4-dichloro-2-fluoronitrobenzene, the fluoride is displaced to give the 2-sulfanyl compound. This compound was purified by flash chromatography as the free amine, an off white solid, mp 119-122°C, R_f 0.20 (EtAc/Heptane 1:20). 1H NMR (DMSO-d6) 5.93 (s, 2H), 6.61 (d, J=8.6, 1H), 7.08 (s,

1H), 7.31 (d of d, J_1=2.1, J_2=8.5, 1H), 7.55 (s, 1H), 7.65 (d, J=1.9, 1H). MS (EI) m/z 337 (M+, 75). Anal. Calcd for $C_{12}H_7Cl_4NS$: C, 42.51; H, 2.08; N, 4.13. Found: C, 42.94; H, 1.97; N, 4.08.

3,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine

2,4-dichlorothiophenol + 3,4,5-trichloronitrobenzene → 4-Nitro-2, 6-dichlorophenyl-(2′, 4′-dichlorophenyl)-sulfide

This compound was recrystallized as the free amine to give a white solid, mp 128-131°C, R_f 0.36 (EtAc/Heptane 1:2). ^{1}H NMR (DMSO-d6) 6.33 (br s, 2H), 6.46 (d, J=8.5, 1H), 6.83 (s, 2H), 7.32 (d of d, J_1=2.1, J_2=8.5, 1H), 7.65 (d, J=2.2, 1H). MS (EI) m/z 337 (M+, 77). Anal. Calcd for $C_{12}H_7Cl_4NS$: C, 42.51; H, 2.08; N, 4.13. Found: C, 42.15; H, 2.10; N, 4.01.

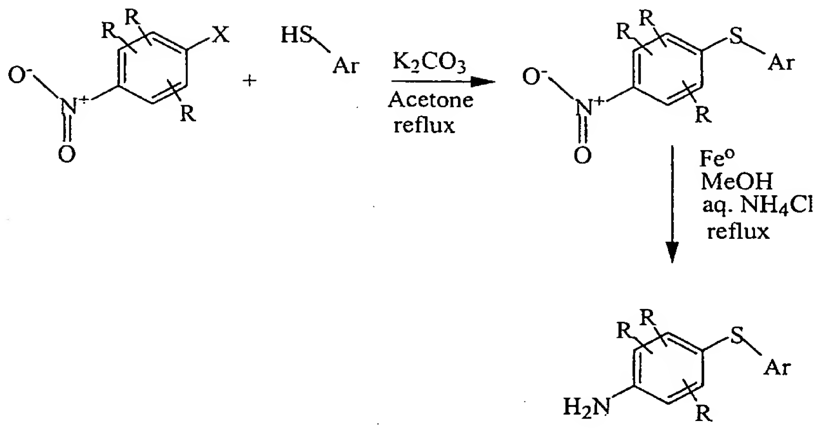
2,3-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine

2,4-dichlorothiophenol + 2,3,4-trinitrobenzene → 2,3-dichloro-4-(2,4-dichlorophenylthio)-nitrobenzene

This compound was purified by flash chromatography as the free amine to give a white solid, mp 116-119°C, R_f 0.33 (EtAc/Heptane 1:4 w/ 1% TEA). ^{1}H NMR (DMSO-d6) 6.09 (s, 2H), 6.47 (d, J=8.7, 1H), 6.86 (d, J=8.7, 1H), 7.32 (d of d, J_1=2.3, J_2=8.7, 1H), 7.46 (d, J=8.7, 1H), 7.69 (d, J=2.2, 1H). MS (EI) m/z 337 (M+, 71). Anal. Calcd for $C_{12}H_7Cl_4NS$: C, 42.51; H, 2.08; N, 4.13. Found: C, 42.83; H, 2.02; N, 4.06.

B. <u>General Synthesis Method B</u>

A General Synthesis Method B is diagramed schematically below:



wherein X is a halogen, preferably chloro.

The following compounds were prepared using General Synthesis Method B:

4-Nitro-2-chlorophenyl-(2',4'-dimethylphenyl)-sulfide

4-Amino-2-chlorophenyl-(2',4'-dimethylphenyl)-sulfide hydrochloride

4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide hydrochloride

4-amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide

4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide

Bis-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-amino-5-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide

4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide

4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide

5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

The initial step in the General Synthesis Method B is illustrated by preparation of the intermediate compound described immediately below.

4-Nitro-2-chlorophenol-(2',4'dimethylphenyl)-sulfide

2,4-Dimethylthiophenol (1.0 g) and 3,4-dichloronitrobenzene (1 eq) were added to 100 ml acetone containing K_2CO_3 (5 g). The mixture was refluxed for 24 hr. After cooling to room temperature, the mixture was filtered and the acetone removed using a rotary vacuum. The resulting residue was dissolved in a small volume of CH_2Cl_2 and filtered. The CH_2Cl_2 was then removed using a rotary vacuum. The resulting residue was treated with methanol (MeOH), which caused the product to precipitate. The yellow solid product was then collected by filtration and dried at 40°C in a vacuum oven for 24 hr. The product yield was 66%, and the melting point was 128-130°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

The following compounds were prepared using an initial step generally in accordance with the above illustrative intermediate.

4-Amino-2-chlorophenyl-(2',4'-dimethylphenyl)-sulfide Hydrochloride

2-Chloro-4-nitrophenyl-(2',4'-dimethylphenyl)-sulfide (0.85 g) and iron powder in MeOH (300 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:4). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The off-white solid product was collected by filtration and dried at 50°C in a vacuum oven for 24 hr. The product yield was 57%, and the melting point was 180-185°C (decomposition). Standard analytical techniques, including proton

NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide (1.0 g) and iron powder in MeOH (300 ml) were added to 5 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform:hexanes (1:4) and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:1). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The white solid product was collected by filtration and dried at 60°C in a vacuum oven for 24 hr. The product yield was 71%, and the melting point was 91-93°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide Hydrochloride

4-Nitro-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide and iron powder in MeOH (300 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:5). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The colorless solid product was collected by filtration and dried at 50°C in a vacuum oven for 24 hr. The product yield was 88%, and the melting point

was 187-190°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(2',4',6;-trichlorophenyl)-sulfide (0.6 g) and iron powder in MeOH (250 ml) were added to 32 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:3). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The white solid product was collected by filtration and dried at 40°C in a vacuum oven for 24 hr. The product yield was 72%, and the melting point was 109-111°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide (1.02 g) and iron powder in MeOH (250 ml) were added to 60 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:7). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The off-white solid product was collected

by filtration and dried at 50°C in a vacuum oven for 24 hr. The product yield was 59%, and the melting point was 86-88°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide (1.0 g) and iron powder in MeOH (250 ml) were added to 40 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:7). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The colorless solid product was collected by filtration and dried at 70°C in a vacuum oven for 24 hr. The product melting point was 103-105°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide

4-Nitro-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide (1.5 g) and iron powder in MeOH (250 ml) were added to 80 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:7). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The colorless solid product was collected

by filtration and dried at 60°C in a vacuum oven for 24 hr. The product yield was 97%, and the melting point was 96-98°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

***Bis*-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide**

4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide and iron powder in MeOH (125 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:7). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The off-white solid product was collected by filtration and dried at 60°C in a vacuum oven for 24 hr. The product yield was 66%, and the melting point was 113-115°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-nitrophenyl-(2',4'-dichlorophenyl)-sulfide and iron powder in MeOH (50 ml) were added to 50 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered, and the solvent was then removed from the filtrate using a rotary evaporator. The product was obtained by stirring the residue with 75 ml of distilled water (dH_2O). The colorless solid precipitate was collected by filtration. The product yield was 83%, and the melting point was 105-107°C. Standard analytical

techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide and iron powder in MeOH (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform-hexane (1:4). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The off-white solid product was collected by filtration and dried at 60°C in a vacuum oven for 24 hr. The product yield was 76%, and the melting point was 170-175°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide (0.2 g) and iron powder in MeOH (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (2:3). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The pale yellow solid product was collected by filtration and dried at 60°C in a vacuum oven for 24 hr. The product yield was

58%, and the melting point was 133-135°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-nitro-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide (0.2 g) and iron powder in methanol (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:4). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The brown solid product was collected by filtration and dried at 50°C in a vacuum oven for 24 hr. The product yield was 16%, and the melting point was 65-70°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

2-Chloro-4-amino-5-N-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-nitro-5-N-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide (0.9 g) and iron powder in MeOH (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved mixture to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:2). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The off-white solid product was collected

by filtration and dried at 50°C in a vacuum oven for 24 hr. The product melting point was 153-155°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide

4-Nitro-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide (0.6 g) and iron powder in MeOH (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:1). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The product was collected by filtration and dried at 50°C in a vacuum oven for 24 hr. The product melting point was not determined because it was a colorless oil. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide

4-Nitro-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide and iron powder in MeOH (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (1:1). Fractions containing the product were combined and the solvents removed using a

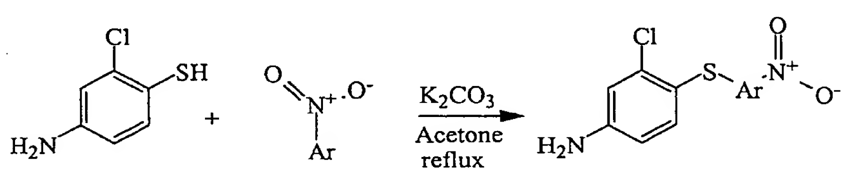
rotary vacuum. The pale yellow solid product was collected by filtration and dried at 30°C in a vacuum oven for 24 hr. The product yield was 55%, and the melting point was 101-102°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

3-Chloro-5-nitrophenyl-(2',4'-dichlorophenyl)-sulfide (1.0 g) and iron powder in MeOH (250 ml) were added to 20 ml of an aqueous solution of NH_4Cl, in a molar ratio of 1:3:5 for substrate, iron powder and NH_4Cl, respectively. The mixture was stirred mechanically under reflux conditions overnight. The solvent was removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:2). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The product was collected by filtration and dried at 25°C in a vacuum oven for 24 hr. The product yield was 16%, and the melting point was not determined because it was a brown oil. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

C. General Synthesis Method C

A General Synthesis Method C is diagramed schematically below:



The following compounds were prepared by General Synthesis Method C:

4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide

4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide

4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide

2-Chloro-4-aminothiophenol (2.0 g) and 3,4-dichloronitrobenzene (1 eq.) were added to 300 ml of acetone containing K_2CO_3 (20 g). The mixture was refluxed for 24 hr at 60°C. After cooling to room temperature, the mixture was filtered and the acetone removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The chloroform was then removed using a rotary vacuum. The resulting residue was triturated with MeOH, which caused the product to precipitate. The precipitate was collected by filtration and the yellow solid product dried at 80°C in a vacuum oven for 24 hr. The product yield was 59%, and the melting point was 135-136°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide

2-Chloro-4-aminothiophenol (2.0 g) and 2,5-dichloronitrobenzene (1 eq.) were added to 300 ml of acetone containing K_2CO_3 (20 g). The mixture was refluxed for 24 hr at 60°C. After cooling to room temperature, the mixture was filtered and the acetone removed using a rotary vacuum. The resulting residue was dissolved in a small volume of CH_2Cl_2 and filtered. The CH_2Cl_2 was then removed using a rotary vacuum. The resulting residue was triturated with MeOH, which caused the product to precipitate. The precipitate was collected by filtration and the yellow solid product dried at 60°C in a vacuum oven for 24 hr. The product yield was 57%, and the melting point was 191-193°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide

2-Chloro-4-aminothiophenol (0.474 g) and 6-chloro-5-nitroquinoline (1 eq.) were added to 250 ml of acetone containing K_2CO_3 (20 g). The mixture was refluxed for 24 hr at 60°C. After cooling to room temperature, the mixture was filtered and the acetone removed using a rotary vacuum. The resulting residue was dissolved in a small volume of chloroform and filtered. The chloroform was then removed using a rotary vacuum. The resulting residue was triturated with MeOH, which caused the product to precipitate. The product was obtained using preparative chromatography by applying the dissolved residue to a small column packed with silica gel (70-230 mesh), followed by elution using a solvent system of chloroform:hexane (3:2). Fractions containing the product were combined and the solvents removed using a rotary vacuum. The yellow solid product was collected by filtration and dried at 50°C in a vacuum oven for 24 hr. The product yield was 62%, and the melting point was 129-131°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

D. Specific Synthesis Procedures

Full preparative methods are provided for the following compounds:

1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene

3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine Hydrochloride

6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole

1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene

0.165 g (2.1 eq, 1.35 mmol) 4-dimethylaminopyridine (4-DMAP) was placed into a dry flask under an atmosphere of nitrogen and dissolved in 5 ml anhydrous tetrahydrofuran (THF). 2 ml of acetic anhydride was added followed by 0.220 g (1 eq, 0.643 mmol) 3-chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride. This was stirred for 18 hrs. The mixture was then diluted with 75 ml ether and washed with sat. $NaHCO_3$ (3 x 50 ml), 0.3 N HCl (3 x 30 ml) and saturated NaCl (2 x 30 ml), dried over Na_2SO_4, filtered, and the solvent stripped on a rotavapor.

Flash chromatography (1.9 x 27 cm, EtAc/Heptane (1:1)) afforded 0.135 g (61% yield) of a white solid, mp 167-169°C, R_f 0.25 (EtAc/Heptane 1:1). ^{1}H NMR (DMSO-d6) 2.07 (s, 3H), 6.59 (d of d, J_1=1.3, J_2=7.9, 1H), 7.24 (t, J=8.1, 1H), 7.46 (d of d, J_1=1.4, J_2=8.1, 1H), 7.55 (m, 2H), 8.04 (d, J=1.9, 1H), 10.35 (s, 1H). MS (EI) m/z 345 (M+, 82). Anal. Calcd for $C_{14}H_{10}Cl_3NOS$: C, 48.51; H, 2.91; N, 4.04. Found: C, 48.29; H, 2.88; N, 3.92.

3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine Hydrochloride

0.19 g (0.67 mmol) 3-Methoxy-4-(2,3-dichlorophenylsulfanyl) phenylmine was placed into a dry flask under an atmosphere of nitrogen, dissolved in 10 ml dry CH_2Cl_2, and cooled to -78°. 0.32 ml (5 eq., 3.3 mmol) boron tribromide was added drop-wise, with stirring. The cold bath was removed and this was left to react for 20 hrs. The solution was again cooled to -78°, and then 10 ml MeOH was added drop-wise. This was allowed to warm to room temperature and was stirred for 1 hr. The solvent was stripped on a rotavapor and the resulting oil was twice taken up in 10 ml MeOH and again stripped. The material was again taken up in 10 ml MeOH and then diluted with 100 ml EtAc. The white precipitate that formed was removed by filtration, and then the filtrate was washed with saturated NaCl (3 x 50 ml), dried over Na_2SO_4, filtered, and the solvent stripped by rotary evaporation. The resulting oil was purified by flash chromatography (2.9 x 28 cm, EtAc/Heptane (1:3)) and was then dissolved in 25 ml of ether and precipitated as the HCl salt by the addition of 5 ml 1 N HCl in ether. This was collected by filtration, washed with ether, and dried (90 oC, 3 hr, 0.3 mm Hg) to gave 0.17 g (80% yield) of an off-white solid, mp 222°C (dec), R_f 0.49 (EtAc/Heptane 1:1). ^{1}H NMR (DMSO-d6) 6.56-6.61 (m, 2H), 6.79 (d, J=1.9, 1H), 6.90 (br hump), 7.19 (t, J=8.1, 1H), 7.27 (d, J=8.2, 1H), 7.38 (d of d, J_1=1.4, J_2=8.1, 1H), 10.37 (br s, 1H). MS (EI) m/z 285 M+ [100]. Anal. Calcd for $C_{12}H_9Cl_2NOS{\cdot}HCl$: C, 44.67; H, 3.12; N, 4.34. Found: C, 44.53; H, 2.91; N, 4.17.

6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole

4-Chloro-2-nitro-5-(2,4-dichlorophenylsulfanyl)-phenylamine was prepared by the general procedure with the exception that it was purified as the free

amine by flash chromatography. 1.37 g (3.93 mmol) of the free amine was added to 10 ml DMF and 10 ml of EtOH. 4.43 g (5 eq, 19.7 mmol) tin chloride dihydrate was added followed by 10 ml concentrated HCl. This was heated to 60°C for 20 hrs. The reaction was allowed to cool to ambient temperature, and was then diluted with 30 ml water, and brought to pH 12 by the addition of 30 ml 5 N NaOH. This mixture was twice extracted with 150 ml ether. The combined organics were washed with 100 ml sat. $NaHCO_3$ and 2 x 100 ml sat. NaCl, dried over Na_2SO_4 and filtered. 40 ml heptane was then added, and this was concentrated on a rotavapor and dried in vacuo (100°C, 2 hr, 0.3 mm Hg) to yield 1.06 g (82%) of an analytically pure white solid, mp 206-208°C, Rf 0.51 (CH_2Cl_2/MeOH (9:1) w/ 1% TEA). ^{1}H NMR (DMSO-d6) 6.63 (d, J=8.6, 1H), 7.28 (d of d, J_1=2.2, J_2=8.7, 1H), 7.69 (d, J=2.2; 1H), 7.86 (br s, 1H), 7.93 (s, 1H), 8.38 (s, 1H), 12.80 (s, 1H). MS (EI) m/z 328 (M+, 97). Anal. Calcd for $C_{13}H_7Cl_3N_2S$: C, 47.37; H, 2.14; N, 8.50. Found: C, 47.40; H, 2.04; N, 8.32.

E. Specific Synthesis Protocols

The following compounds were prepared by methods as described below.

4-Methylamino-2,2',4'-trichlorodiphenylsulfide

4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide

4-Aminomethyl-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

4-Methylamino-2,2',4'-trichlorodiphenylsulfide

4-Amino-2,2',4'-trichlorodiphenyl-sulfide (0.305 g, 1.0 mmol) was added to 15 ml of formic acid. The mixture was stirred for 8 hr, after which 0.23 ml of 37% formaldehyde was added and the mixture refluxed for 8 hr. The solvent was then removed using a rotary evaporator. The resulting residue was then applied to a small column containing silica gel (70-230 mesh). The product was then eluted from the column using chloroform. The solvent was removed from the eluate using a rotary evaporator and the pale yellow solid product collected. The product yield was 44%, and the melting point was 211°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were

employed to characterize the product.

4-Nitro-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide

4-Amino-2,2'-dichloro-4'-nitrodiphenylsulfide (1.0 g, 3.17 mmol) was warmed in 15 ml of acetic anhydride containing a trace of *p*-toluenesulfonic acid. The mixture was allowed to stand for 1 hr, after which the solvent was removed using a rotary evaporator. The residue was dissolved in EtAc and poured over a small column of silica gel (70-230 mesh). The filtrate was collected, evaporated to dryness, and recrystallized from acetonitrile to give a yellow solid product. The product yield was 97%, and the melting point was 163-165°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Nitro-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide (1.0 g, 3.17 mmol) was added to a suspension of 60% sodium hydride (1.43 g) in 250 ml of THF at 0°C. Iodomethane (0.2 ml, in 20 ml of THF) was then added, and the mixture stirred at room temperature for 48 hr. The mixture was then applied to Analtech silica gel plates. Each plate had a thickness of 1000 microns of silica. The two products of the reaction, i.e., 4-nitro-2-chlorophenyl-(4'-methylamino-2'-chlorophenyl)-sulfide and 4-nitro-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide, were eluted using a 1:1 mixture of $CHCl_3$ and hexane. The slower eluting band corresponded to the former and the faster eluting band corresponded to the latter. After collecting the bands, the compounds were dissolved in $CHCl_3$. The solvent was removed by rotary evaporation to provide a yellow solid product. The yield of the desired product was 36%, and the melting point was 138-139°C. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

4-Aminomethyl-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-cyanopheny-(2',4'-dichlorophenyl)-sulfide (1.0 g, 3.18

mmol) was added to 200 ml of THF under nitrogen at 0°C. Lithium aluminum hydride (0.24 g) was then added to the solution in portions. The mixture was then allowed to stand for 2 hr at 0°C, after which 1 ml of 20% NaOH was added to the mixture, followed by 1 ml of dH_2O. The solution was then filtered, and solvents were removed using a rotary evaporator. The resulting residue was then applied to a small column containing silica gel (70-230 mesh). The column was washed with a 1:1 mixture of chloroform and hexane, after which the product was eluted using a rotary evaporator, and the product was obtained as a colorless oil. The product yield was 40%, and the melting point was not determined. Standard analytical techniques, including proton NMR spectroscopy, elemental analysis and thin layer chromatography, were employed to characterize the product.

F. Other compounds

Other compounds synthesized in accordance with the general synthetic methods include the following.

Table II

4-Nitro-2-chlorophenyl-(2′,4′-dimethylphenyl)-sulfide
4-Nitro-2-chlorophenyl-(2′-methyl-4′-chlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(2′, 4′-difluorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(2′, 4′, 6′-trichlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(3′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide
2-Chloro-4-nitrophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(4′-acetamido-2′-chlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(4′-dimethylamino-2′-chlorophenyl)-sulfide
2-Chloro-4-nitro-5-methylaminophenyl-(2′, 4′-dichlorophenyl)-sulfide
2-Chloro-4-nitro-5-morpholinophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-trifluoromethylphenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-fluorophenyl-(2′, 4′-dichlorophenyl)-sulfide
3-Chloro-5-nitrophenyl-(2′, 4′-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(1-naphthyl)-sulfide
4-Nitro-2-methylphenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-bromophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2, 5-dichlorophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2, 6-dichlorophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-chloro-5-fluorophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2, 3-dichlorophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(4′-chloro-2′-aminophenyl)-sulfide
4-Nitro-5-acetamido-2-chlorophenyl-(2′, 4′-dichlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(4′-methylamino-2′-chlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(4′-benzylamino-2′-chlorophenyl)-sulfide
4-Nitro-2-chlorophenyl-(4′-dibenzylamino-2′-chlorophenyl)-sulfide
4-Nitro-5-phenylsulfo-2-chlorophenyl-(2′, 4′-dichlorophenyl)-sulfide
3-Nitro-5-chlorophenyl-(2′, 4′-dichlorophenyl)-sulfide

Example 4

Cell-Based Assay Results

Compounds of the invention displayed activity in the cell-based assays described above as set out in Table III below which provides μM IC_{50} values for inhibition of LFA-1 binding to ICAM-1 and ICAM-3 where tested. Paired values (X/Y) indicate inhibition in the absence and presence of IL-8. Multiple paired values (W/X; Y/Z) indicate repeated experiements. Dashes (--/X) indicate the experiment was not performed. "NT" indicates that the compound was not tested in a particular assay.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

TABLE III

Compound	LFA1/ICAM-1 IC50 -/+ IL-8	LFA-1/ICAM-3 IC50 -/+ IL-8
3-Chloro-4-(2-chlorophenylsulfanyl)-phenylamine hydrochloride	7.6/4.7; 17.7/16.3	NT
3-Chloro-4-(2-naphthylsulfanyl)-phenylamine hydrochloride	7.8/7.2; 10.0/9.3	NT
3-Chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride	1.1/0.7; 3.4/3.9	1.8/1.0
3-Chloro-4-(2,4,5-trichlorophenylsulfanyl)-phenylamine hydrochloride	7.5/12.5; 18.9/22.6	NT
3-Chloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine Hydrochloride	1.6/1.9; 3.0/4.1; 5.0/6.0	1.2/1.4
3-Methoxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine	--/7.5; 8.7/7.0	NT
5-Amino-2-(2,3-dichlorophenylsulfanyl)-acetophenone hydrochloride	--/20	NT
4-(2,3-dichlorophenylsulfanyl)-phenylamine	--/8.0; 8.5/7.6	NT
3-Chloro-4-(1-naphthylsulfanyl)-phenylamine hydrochloride	--/31	NT
3-Methyl-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride	7.9/8.2; 7.0/6.5	NT
1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene	--/29.5	NT
4-Methylamino-2,2',4'-trichlorodiphenylsulfide	34.0/>40	NT
3-Bromo-4-(2,4-dichlorophenylsulfanyl)-phenylamine Hydrochloride	4.0/7.5; 2.6/3.1	NT
3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine Hydrochloride	14.2/14.2	NT
6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole	27.8/>41	NT
4-Amino-2-chlorophenyl-(2',4'-dimethylphenyl)-sulfide Hydrochloride	12.6/23.0	NT
2,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine Hydrochloride	3.3/3.9; 3.6/3.1; 2.2/4.0	1.1/1.1
4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide	13.4/22.6	NT
4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide Hydrochloride	16.5/17.8	NT
4-Amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide	8.7/12.4	NT
4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide	29.0/36.0	NT
4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide	10.0/12.0	NT
4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide	6.7/7.8; 6.0/5.6	2.5/2.3; 1.1/3.0
4-Amino-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide	17.4/14.5	NT
4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide	9.3/13.5; 7.6/7.3	NT
Bis-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide	35.5/30.5	NT
4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide	6.0/6/7; 5.9/4.2	2.1/2.0
4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide	17.0/29.0	NT

4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide	6.0/7.3; 3.8/7	2.5/3.8
4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide	9.7/9.2	NT
2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide	17.7/31.6	NT
2-Chloro-4-amino-5-N-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide	35.7/24.3	NT
4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide	7.5/6.6	NT
4-Aminomethyl-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide	>40/18.7	NT
4,5-Dichloro-2-(2,4-dichlorophenylsulfanyl)-phenylamine	31.5/35.0; 33.7/36	NT
3,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine	3.9/5.0	NT
2,3-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine	27.5/27.5	NT
4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide	4.8/5.9	NT
5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide	31.8/32.9	NT
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine	10.0/8.3; 7.8/8.8	3.0/3.5
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine	1.0/1.5	0.5/0.3
4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide	29.0/40.0	NT
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine-sulfide	>40/31.0	NT

WHAT IS CLAIMED IS:

1. A compound selected from the group consisting of:

3-Chloro-4-(2-chlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2-naphthylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4,5-trichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Methoxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine
5-Amino-2-(2,3-dichlorophenylsulfanyl)-acetophenone hydrochloride
4-(2,3-dichlorophenylsulfanyl)-phenylamine
3-Chloro-4-(1-naphthylsulfanyl)-phenylamine hydrochloride
3-Methyl-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene
4-Methylamino-2,2',4'-trichlorodiphenylsulfide
3-Bromo-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole
4-Amino-2-chlorophenyl-(2'4'-dimethylphenyl)-sulfide hydrochloride
2,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide hydrochloride
4-amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(3',4-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide
Bis-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide

4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide

2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide

2-Chloro-4-amino-5-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide

4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide

4-Aminomethyl-2-chlorophenyl-(2'4'-dichlorophenyl)-sulfide

4,5-Dichloro-2-(2,4-dichlorophenylsulfanyl)-phenylamine

3,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine

2,3-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine

4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide

5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(2',4'-dimethylphenyl)-sulfide

4-Nitro-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(2', 4'-difluorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(2', 4', 6'-trichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(3', 4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide

2-Chloro-4-nitrophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide

2-Chloro-4-nitro-5-methylaminophenyl-(2', 4'-dichlorophenyl)-sulfide

2-Chloro-4-nitro-5-morpholinophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-trifluoromethylphenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-fluorophenyl-(2', 4'-dichlorophenyl)-sulfide

3-Chloro-5-nitrophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(1-naphthyl)-sulfide

4-Nitro-2-methylphenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-bromophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2, 5-dichlorophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2, 6-dichlorophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-chloro-5-fluorophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2, 3-dichlorophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-chloro-2'-aminophenyl)-sulfide

4-Nitro-5-acetamido-2-chlorophenyl-(2', 4'-dichlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-methylamino-2'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-benzylamino-2'-chlorophenyl)-sulfide

4-Nitro-2-chlorophenyl-(4'-dibenzylamino-2'-chlorophenyl)-sulfide

4-Nitro-5-phenylsulfo-2-chlorophenyl-(2', 4'-dichlorophenyl)-sulfide

3-Nitro-5-chlorophenyl-(2', 4'-dichlorophenyl)-sulfide

2. A pharmaceutical composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier.

3. A pharmaceutical composition comprising a compound selected from the group consisting of:

3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine

1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone

1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone oxime

5-Trifluoromethyl-2-phenylsulfanyl-benzonitrile

1-(3,5-dichlorophenyl)-3-phenylsulfanyl-pyrrolidine-2,5-dione

Bis-2,4,6-Trinitrophenyl-sulfide

2-Methyl-1-(2-*o*-tolylsulfanyl-phenyl)-1*H*-pyrrole

3-[2-(4-Chloro-2-nitro-phenylsulfanyl)-phenylamino-3*H*-isobenzofuran-1-one

4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine

2-Nitro-4-chlorophenyl-(2'aminophenyl)-sulfide

6-Amino-2-chlorophenyl-(4'-methylphenyl)-sulfide

4-Nitrophenyl-(2'-chlorophenyl)-sulfide

2, 4-Dinitrophenyl-(4'-chlorophenyl)-sulfide

4-Aminophenyl-(2'-chlorophenyl)-sulfide

2, 4-Diaminophenyl-(4'-isopropylphenyl)-sulfide

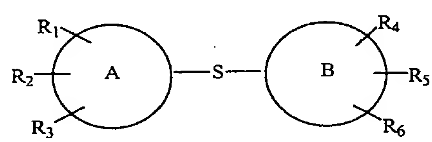
4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine sulfide
4-Amino-2-chlorophenyl-(2′-nitro-4′-chlorophenyl)-sulfide

4. A method for treating an inflammatory disorder, comprising a step of administering to a mammal an amount of the pharmaceutical composition of claim 2 or 3 sufficient to inhibit binding of LFA-1 to a natural ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1.

5. A method for inhibiting LFA-1 binding to an ICAM that binds LFA-1 comprising the step of contacting LFA-1 with a diaryl sulfide.

6. The method according to claim 5 wherein the diaryl sulfide is substituted.

7. The method of claim 5 wherein the diaryl sulfide is a compound represented by the general structural formula (I):



(I)

wherein A and B, independently, are aryl groups selected from the group consisting of 5- and 6-membered aromatic rings, including, but not limited to, phenyl, thienyl, furyl, pyrimidinyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrrolyl, and pyridazinyl;

R_1, R_2 and R_3, independently, are selected from the group consisting of

hydrogen,

-R_a, wherein R_a is hydrogen or an alkyl group containing one to six saturated straight or branched chain carbon atoms (C_{1-6} alkyl),

-O-R_a,

-halo, wherein halo is Cl, F, Br, or I,

-NR_bR_c, where R_b and R_c, independently, are H, C_{1-6} alkyl, or -CH_2-aryl,

-NO_2,

-C(=O)R_a,

-CN,

-perfluoroR_a, such as trifluoromethyl,

-N-C(=O)R_a,

-$(CH_2)_n$-NR_bR_c, wherein n is an integer 1 to 6,

a 5- or 6-membered heterocyclic ring, either aliphatic or aromatic, containing one or more of O, N, or S, optionally substituted, such as morpholino, and

-S-aryl, wherein aryl is a 5- or 6-membered aromatic ring, optionally substituted;

and R_4, R_5 and R_6, independently, are selected from the group consisting of

hydrogen,

-R_a,

-O-R_a,

-halo,

-NR_bR_c,

-NO_2,

-C(=O)R_a,

-CN,

-perfluoroR_a,

-N-C(=O)R_a,

-$(CH_2)_n$-NR_bR_c, and

-a 5- or 6-membered heterocyclic ring, aliphatic or aromatic, containing one or more of O, N, or S, and optionally substituted,

-S-aryl, or wherein

R_4 and R_5 are taken together to form a 5- or 6-membered aromatic ring, optionally containing one or more of O, N, or S in the ring, optionally substituted.

8. The method according to claim 7 wherein the diaryl sulfide is selected from the group consisting of:

3-Chloro-4-(2-chlorophenylsulfanyl)-phenylamine hydrochloride
4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide
3-Chloro-4-(2-naphthylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4,5-trichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
3-Methoxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine
5-Amino-2-(2,3-dichlorophenylsulfanyl)-acetophenone hydrochloride
4-(2,3-dichlorophenylsulfanyl)-phenylamine
3-Chloro-4-(1-naphthylsulfanyl)-phenylamine hydrochloride
3-Methyl-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene
4-Methylamino-2,2',4'-trichlorodiphenylsulfide
3-Bromo-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole
4-Amino-2-chlorophenyl-(2'4'-dimethylphenyl)-sulfide hydrochloride
2,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide hydrochloride
4-Amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide

4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide
Bis-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide
4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide
2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide
2-Chloro-4-amino-5-N-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine sulfide
4-Aminomethyl-2-chlorophenyl-(2', 4'-dichlorophenyl)-sulfide
4,5-Dichloro-2-(2,4-dichlorophenylsulfanyl)-phenylamine
3,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine
2,3-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine
4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide
5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone oxime
5-Trifluoromethyl-2-phenylsulfanyl-benzonitrile
1-(3,5-dichlorophenyl)-3-phenylsulfanyl-pyrrolidine-2,5-dione
Bis-2,4,6-Trinitrophenyl-sulfide
2-Methyl-1-(2-*o*-tolylsulfanyl-phenyl)-1*H*-pyrrole
3-[2-(4-Chloro-2-nitro-phenylsulfanyl)-phenylamino-3*H*-isobenzofuran-1-one
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
2-Nitro-4-chlorophenyl-(2'aminophenyl)-sulfide
6-Amino-2-chlorophenyl-(4'-methylphenyl)-sulfide
4-Nitrophenyl-(2'-chlorophenyl)-sulfide

2, 4-Dinitrophenyl-(4'-chlorophenyl)-sulfide

4-Aminophenyl-(2'-chlorophenyl)-sulfide

2, 4-Diaminophenyl-(4'-isopropylphenyl)-sulfide

4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide

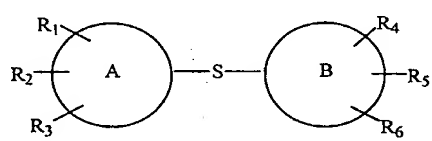
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine-sulfide

9. A method for treating an inflammatory disorder arising from LFA-1 binding to a natural ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1, comprising administering to a mammal in need thereof a compound that competes with 3-chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine for binding to LFA-1 in an amount sufficient to inhibit binding of the natural ligand to LFA-1.

10. The method of claim 9 wherein the compound is a diaryl sulfide.

11. The method of claim 10 wherein the diaryl sulfide is substituted.

12. The method according to claim 10 wherein the diaryl sulfide is a compound represented by the general structural formula (I):



(I)

wherein A and B, independently, are aryl groups selected from the group consisting of 5- and 6-membered aromatic rings, including, but not limited to, phenyl, thienyl, furyl, pyrimidinyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrrolyl, and pyridazinyl;

R_1, R_2 and R_3, independently, are selected from the group consisting of

hydrogen,
-R_a, wherein R_a is hydrogen or an alkyl group containing one to six saturated straight or branched chain carbon atoms (C_{1-6} alkyl),
-O-R_a,
-halo, wherein halo is Cl, F, Br, or I,
-NR_bR_c, where R_b and R_c, independently, are H, C_{1-6} alkyl, or -CH_2-aryl,
-NO_2,
-C(=O)R_a,
-CN,
-perfluoroR_a, such as trifluoromethyl,
-N-C(=O)R_a,
-$(CH_2)_n$-NR_bR_c, wherein n is an integer 1 to 6,
a 5- or 6-membered heterocyclic ring, either aliphatic or aromatic, containing one or more of O, N, or S, optionally substituted, such as morpholino, and
-S-aryl, wherein aryl is a 5- or 6-membered aromatic ring, optionally substituted;

and R_4, R_5 and R_6, independently, are selected from the group consisting of

hydrogen,
-R_a,
-O-R_a,
-halo,
-NR_bR_c,
-NO_2,
-C(=O)R_a,
-CN,
-perfluoroR_a,
-N-C(=O)R_a,
-$(CH_2)_n$-NR_bR_c, and

-a 5- or 6-membered heterocyclic ring, aliphatic or aromatic, containing one or more of O, N, or S, and optionally substituted,

-S-aryl, or wherein

R_4 and R_5 are taken together to form a 5- or 6-membered aromatic ring, optionally containing one or more of O, N, or S in the ring, optionally substituted.

13. The method according to claim 12 wherein the diaryl sulfide is selected from the group consisting of:

3-Chloro-4-(2-chlorophenylsulfanyl)-phenylamine hydrochloride
4-Nitro-2-chlorophenyl-(2',3'-dichlorophenyl)-sulfide
3-Chloro-4-(2-naphthylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4,5-trichlorophenylsulfanyl)-phenylamine hydrochloride
3-Chloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
3-Methoxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine
5-Amino-2-(2,3-dichlorophenylsulfanyl)-acetophenone hydrochloride
4-(2,3-dichlorophenylsulfanyl)-phenylamine
3-Chloro-4-(1-naphthylsulfanyl)-phenylamine hydrochloride
3-Methyl-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
1-Acetamido-3-chloro-4-(2,3-dichlorophenylsulfanyl)-benzene
4-Methylamino-2,2',4'-trichlorodiphenylsulfide
3-Bromo-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
3-Hydroxy-4-(2,3-dichlorophenylsulfanyl)-phenylamine hydrochloride
6-Chloro-5-(2,4-dichlorophenylsulfanyl)-1H-benzimidazole
4-Amino-2-chlorophenyl-(2'4'-dimethylphenyl)-sulfide hydrochloride
2,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine hydrochloride
4-Amino-2-chlorophenyl-(2'-methyl-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-difluorophenyl)-sulfide hydrochloride

4-amino-2-chlorophenyl-(2',4',6'-trichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-amino-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-chloro-4'-nitrophenyl)-sulfide
4-Amino-2-chlorophenyl-(2'-nitro-4'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(3',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(3-chloro-5-trifluoromethylpyridyl)-sulfide
Bis-(4,4'-diamino-2,2'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-(4'-acetamido-2'-chlorophenyl)-sulfide
4-Amino-2-chlorophenyl-6-(5-nitroquinolino)-sulfide
4-Amino-2-chlorophenyl-(4'-dimethylamino-2'-chlorophenyl)-sulfide
2-Chloro-4-amino-5-methylaminophenyl-(2',4'-dichlorophenyl)-sulfide
2-Chloro-4-amino-5-N-morpholinophenyl-(2',4'-dichlorophenyl)-sulfide
4-Amino-2-trifluoromethylphenyl-(2',4'-dichlorophenyl)-sulfideIC86405
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine sulfide
4-Aminomethyl-2-chlorophenyl-(2', 4'-dichlorophenyl)-sulfide
4,5-Dichloro-2-(2,4-dichlorophenylsulfanyl)-phenylamine
3,5-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine
2,3-Dichloro-4-(2,4-dichlorophenylsulfanyl)-phenylamine
4-Amino-2-fluorophenyl-(2',4'-dichlorophenyl)-sulfide
5-Amino-3-chlorophenyl-(2',4'-dichlorophenyl)-sulfide
3-Chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone
1-(3-Nitro-4-phenylsulfanyl-phenyl)-ethanone oxime
5-Trifluoromethyl-2-phenylsulfanyl-benzonitrile
1-(3,5-dichlorophenyl)-3-phenylsulfanyl-pyrrolidine-2,5-dione
Bis-2,4,6-Trinitrophenyl-sulfide
2-Methyl-1-(2-*o*-tolylsulfanyl-phenyl)-1*H*-pyrrole
3-[2-(4-Chloro-2-nitro-phenylsulfanyl)-phenylamino-3*H*-isobenzofuran-1-one
4-(Benzothiazol-2-ylsulfanyl)-3-chloro-phenylamine
2-Nitro-4-chlorophenyl-(2'aminophenyl)-sulfide

6-Amino-2-chlorophenyl-(4′-methylphenyl)-sulfide
4-Nitrophenyl-(2′-chlorophenyl)-sulfide
2, 4-Dinitrophenyl-(4′-chlorophenyl)-sulfide
4-Aminophenyl-(2′-chlorophenyl)-sulfide
2, 4-Diaminophenyl-(4′-isopropylphenyl)-sulfide
4-Nitro-2-chlorophenyl-(2′,3′-dichlorophenyl)-sulfide
4-Amino-2-chlorophenyl-2-(5-nitro-3-bromo)-pyridine-sulfide

14. A method of inhibiting binding of LFA-1 to a natural ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1, comprising contacting a cell that expresses LFA-1 on its surface with a compound that competes with 3-chloro-4-(1-chloro-naphthalen-2-ylsulfanyl)-phenylamine for binding to LFA-1 in an amount that is sufficient to inhibit binding to LFA-1 to the natural ligand.

15. The method according to claim 9 or 14, wherein the ligand is ICAM-1 or ICAM-3.

16. A method to identify a negative regulator of LFA-1 binding to a natural ligand thereof that competes with ICAM-1 or ICAM-3 for binding to LFA-1 comprising the steps of:

a) contacting LFA-1 with an activator of LFA-1 binding;
b) measuring LFA-1 binding with the natural ligand in the presence and absence of a test compound; and
c) identifying the test compound as a negative regulator when decreased LFA-1 binding to the ligand is detected in the presence of the test compound.

17. The method according to claim 16 wherein the activator is crystal violet.